Uncovering novel drug targets of polyprotein precursors of SARS-CoV2

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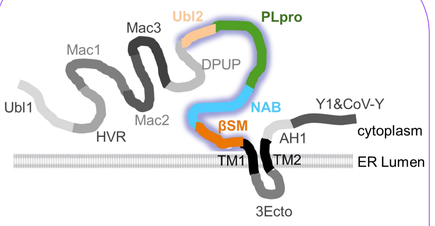
SARS-CoV2 NSP3 (1945 aa)



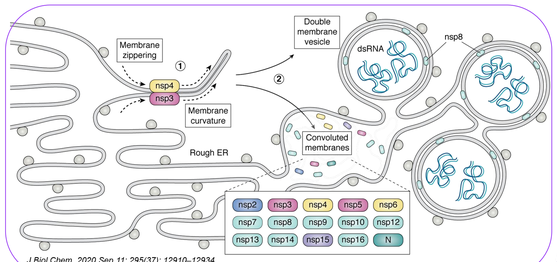
- NSP3 is the largest polyprotein precursor
- Responsible for cleavage of viral proteins
- Involved in vesicles formation crucial for replication
- Innate immune system suppression

Introduction

The SARS-CoV2's genome translates into two polyproteins, pp1a and pp1ab, which are cleaved by two viral proteases (papain-like protease (PLpro) and 3C-like protease (3CLpro)) to produce 16 functional non-structural proteins (NSPs) necessary for viral replication, rendering these proteases potential therapeutic targets. PLpro is located on the largest of all NSPs, NSP3, which together with Nsp4 and Nsp6 are responsible for the formation of double membrane vesicles (DMVs), the central hub for viral RNA replication. Furthermore, multiple of its 16 subdomains, including PLpro, suppress the innate immune response by cleaving off ubiquitin and ISG, which are markers for infected cells. Unlike the classical approach of studying these potential drug targets as individual domains, our project aims at structural and functional studies of PLpro in the polyprotein context, in order to explore novel small molecule binding sites that might provide new leads for antiviral therapeutics.

Objectives

1. Structural and functional studies of PLpro in multidomain context including its neighbor domains such as nucleotide binding domain (NAB) and beta coronavirus specific marker (βSM) using integrated structural biology approach
2. Investigating Ubl2-PLpro-NAB and Ubl2-PLpro-NAB-βSM as potential therapeutic targets using extensive fragment screening



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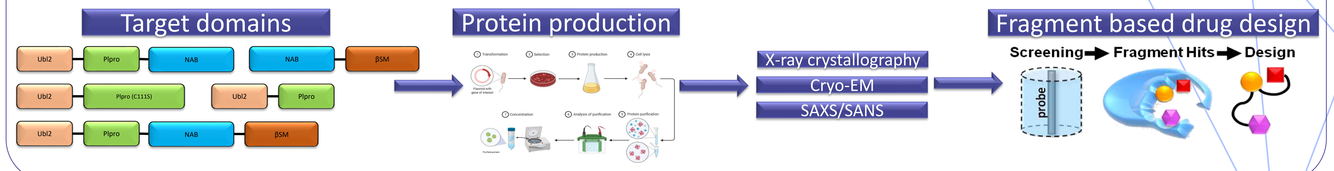
Integrated structural biology approach

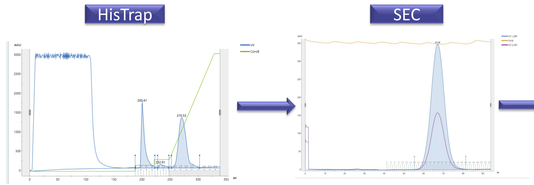


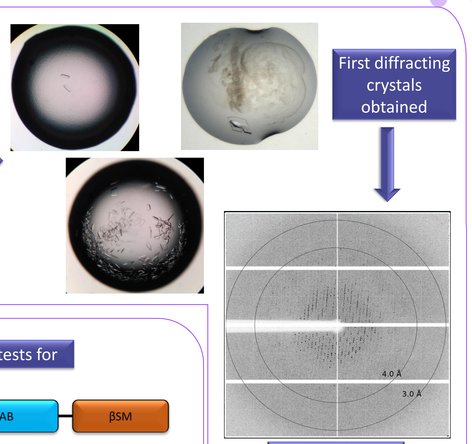
Preliminary results

Thorough purification protocol established for

Ubl2 — Plpro

Ubl2 — Plpro (C111S)



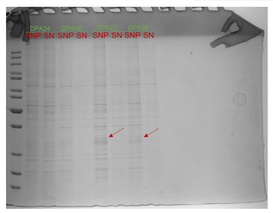


First diffracting crystals obtained

Low resolution

Conditions need to be optimised

Expression tests for NAB βSM



E. coli expression unsuccessful

Expression in SF21 insect cells successful

Expression in Hi5 to be tested next

Expression tests for Ubl2 Plpro NAB βSM

E. coli expression unsuccessful → Expression in SF21 to be tested next

Outlook

Optimisation of the crystal conditions for Ubl2-PlPro WT and its active site mutant (C111S) to achieve high resolution diffracting crystals is underway. This is crucial to perform further fragment screening experiments at Nuvisan to identify potential therapeutic inhibitors. In parallel, expression and purification protocols will be established for the longer constructs including the NAB and βSM domains. Proteins thus produced will be subjected to crystallization trials. To increase our success to obtain the 3D structure of the longer constructs, cryo-EM experiments using phase plate technology will also be attempted. These will be followed by fragment screening trials. SAXS/SANS experiments will be employed to identify potential interactions within these domains in apo-form and with bound inhibitors.

References

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